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Influence of temperature on the embryonic and post-embryonic development of *Scaphoideus titanus* (Hemiptera: Cicadellidae), vector of grapevine Flavescence dorée.

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Influence of temperature on the embryonic and post-embryonic development of *Scaphoideus titanus* (Hemiptera: Cicadellidae), vector of grapevine Flavescence dorée.

The embryonic and post-embryonic development of *Scaphoideus titanus*, the main vector of grapevine Flavescence dorée, was studied under laboratory conditions, at constant temperatures (T=15°, 18°, 20°, 22°, 24°, 27°, and 29°C). The data obtained were fitted to the equation of Brière, and the model was validated against independent field data. The minimum cardinal temperature for eggs ranged from 18° to 20°C, the duration of egg hatching was minimum at T = 24°C, egg hatchability was optimum at 22°C, and very few eggs hatched at T ≥ 27°C. The duration of post-embryonic development clearly shortened as the temperature increased, both overall and within the same life stage, almost half-reducing itself from 18° to 29°C. Within the same temperature tested, the early instars took less time to moult compared to the late ones. The data obtained provided a significant fit with the equation of Brière. Validation was satisfactory, particularly concerning 3rd instar nymphs and adults, which are the key life instars for targeting IPM strategies. The model proposed could be used to predict the development of *S. titanus* in Northwestern Italy for IPM purposes.

Keywords: grapevine; leafhopper; moult; temperature; Brière's equation

1. Introduction

Scaphoideus titanus Ball (Hemiptera: Cicadellidae, Deltocephalinae) is the main vector of grapevine Flavescence dorée (FD), a serious disease caused by phytoplasmas belonging to the 16SrV group (C and D subgroups) (Malembic-Maher et al., 2011). *Scaphoideus titanus* is native to North America, where it is widespread both in Canada and in the U.S. (Barnett, 1976). It was detected in Europe for the first time in the late 1950s in France (Bonfils and Schvester, 1960), and is now widespread in Central Europe, from Portugal to Bulgaria (COST Action FA0807, 2013).

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This species is a grapevine specialist (*Vitis* spp.); it is univoltine, and overwinters in the egg stage, laid under the bark of mainly two-year-old wood (Vidano, 1964). Hatching starts since the middle of May; post-embryonic development includes five nymphal instars (N1-N5) (Vidano, 1964); adults usually appear at the beginning of July, with a peak between the end of July and the middle of August, and persist until mid-end of October, depending on the season (Lessio and Alma, 2004). The nymphs from the third instar onwards acquire phytoplasmas by feeding on infected grapevines (acquisition access period, AAP), and are infective after 28-35 d (latency access period, LAP) meanwhile becoming adults (Bressan et al., 2006).

Scaphoideus titanus is a target for mandatory sprays in many European countries including Italy (Lessio et al., 2011). Depending on the active ingredient (a.i.) to be used, the time of spraying must take into account the life instar of the insect; for instance, insect growth regulators (IGRs) and natural pyrethrum are ineffective against adults. Also, the first spray is often made to target third instar nymphs (N3) which are able to acquire phytoplasmas. Moreover, egg hatching may go on for a long time during the season, exceeding the usual period of insecticidal sprays.

These aspects call for a more detailed knowledge about the seasonal presence of the different life stages of *S. titanus*. Within this frame of mind, agro-meteorological models are useful tools to enhance pest management (Arnold et al., 1976; Severini et al., 2005; Rigamonti et al., 2011). They may depend on biotic factors, such as genetic variability, and non-biotic factors such as air temperature, relative humidity, light radiation, among other factors (Logan et al., 1976). Phenological models are the basis for timing of plant protection measurements against insects (Lieth, 1974). The distribution delay models (DDM) are developed using both phenological and demographic models: the latter describing the variations over time of the size of a population (Manetsch, 1976; Severini and Gilioli, 2002).

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Temperature is probably the most important non-biotic factor influencing insects' life cycles, as it regulates most biochemical reactions (Podolsky, 1984). To predict the relationship between temperature and developmental rate, the first models applied were linear and based on the accumulation of the thermal degree days (Roy et al., 2002). However, because often this response is not linear at limit temperatures (Butturini et al., 1992), non-linear models have been developed (Logan et al., 1976; Brière et al., 1999; Severini and Gilioli, 2002).

Concerning *S. titanus*, Rigamonti et al. (2011) developed a model to predict the development of eggs in the post-dormancy phase and of first instar nymphs (N1) in open fields in Switzerland, whereas N2-N5 were observed at constant temperatures. This model was validated against independent field data, to determine the beginning of egg hatching, N3 appearance, and adult emergence, in order to synchronize the timing of IGR applications against N3. However, some basic information on the response of *S. titanus* to temperature of the different instars was not considered. A separate study on the development of N1 is needed, as the application of insecticides other than IGRs may call for a model that represents the dynamics of *S. titanus* considering age structure.

In Italy, where *S. titanus* was first found in 1963 in Liguria region (Vidano, 1964) and is now widespread in 16 regions, the main problem for pest management is given by the fact that adults enter the vineyards from reservoirs such as uncultivated areas containing wild (American) grapevine plants (Pavan et al., 2012; Lessio et al., 2014), during the latter part of a season. Moreover, the first insecticidal application against nymphs is usually made with active ingredients other than IGRs (for instance neonicotinoids or organophosphates), thus it is important to detect the peak of nymphal presence, to maximize the effect of spraying. Therefore, a basic study on the thermal behaviour of this insect pest for Italy is needed. Our aim was to study the influence of temperature on the developmental rate of the different life stages of *S. titanus* (eggs, nymphal instars, and adults) under laboratory conditions, in order to study the

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embryonic and post-embryonic development of this insect pest and to apply the obtained model in the open field.

2. Materials and methods

2.1. Insect source and rearing

Field samplings were conducted in the district of Dogliani (CN) (44.539529 °N, 7.949967 °E), Piedmont, NW Italy, during 2009-2011. In order to obtain viable eggs of *S. titanus*, grapevine canes (two or more year-old wood) were collected after pruning at the middle of March, in an organic vineyard, cv. Dolcetto, 20 years old, highly infested by the leafhopper according to captures with yellow sticky traps during the previous year (more than 200 adults per trap, from July to September). The canes were stored into a cool chamber (T=5°C, Relative Humidity=65%, Light:Dark=16:8 h) before use.

The developmental rate of *S. titanus* eggs was studied during 2009. The canes were collected on 25 March 2009, and the rearing started on the following day. Rearing cages were set up inside climatic chambers at constant temperatures of 15°, 18°, 20°, 22°, 24°, 27°, and 29° C (one cage per each T tested); the temperature in each chamber was recorded by means of HOBO® data loggers (Onset Computer Corporation, Pocasset, MA) every 15 minutes (RH=65%, L:D=16:8 h). Cages consisted in insect-proof tents made of polyethylene film and nylon mesh (50×50 cm, h 75 cm). A green potted grapevine from nursery stock, cv. Barbera or Chardonnay, h=30 cm, and 0.5-2 kg of grapevine canes, were put into each cage. The cages were inspected every 48 h, until the first eggs hatched; afterwards, they were inspected daily, or at least for six days per week; during each inspection, newly hatched nymphs (N1) were removed from the cages to avoid any mistakes given by double-counting them. Each cage was inspected

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up to 75 days after the last hatching occurred.

The eggs of *S. titanus* were counted on a random subsample of canes, consisting in 30 pieces of wood (total weight 350 g, average length 30 cm). Each piece was inspected by gently removing the bark with a cutter blade, and observing and counting the eggs under a stereomicroscope. Finally, the mean number of eggs per subsample was used to estimate with a proportion the amount of eggs in the wood placed in each cage.

The post-embryonic development of *S. titanus* was studied during 2010-2011. The wood was collected as in the previous year (on 17 March 2010, and 24 March 2011), and stored into the climatic chamber at T=5°C; the rearing started about the end of March, inside the climatic chamber at T=24°, following the same method used during 2009, and using roughly the same amount of wood per cage. When hatching started, N1 cohorts were moved to smaller rectangular-shaped cages (25×25 cm, h 40 cm), made of Plexiglas and insect-proof mesh. A potted grapevine plant was placed inside each cage, as previously described. The cages were kept at constant temperature regimes of 15°, 18°, 20°, 22°, 24°, 27°, and 29°C.

The objective was to calculate the developmental time of at least 25 insects having the same physiological age, per each moult and temperature, overcoming mortality. So every time a cohort of at least 50 N1 was obtained, it was divided into sub-cohorts of N=10, that were put into smaller cages (15 × 15 cm, h 25 cm) to estimate the time of development up to the adult stage. The different sub-cohorts obtained were set up in parallel at the different temperature regimes tested. On the whole, the experiment was started with a number of N1≈500 resulting from 10 different cohorts. In the end, by pooling different sub-cohorts, a data set of N≥25 for each temperature and moult tested was obtained.

Cages were inspected daily in order to check the number and age of *S. titanus* nymphs. After having shaken the grapevine plant to make them fall, insects were removed from the cage with an aspirator and placed into glass tubes (3-5 insects/tube), anesthetized with carbon dioxide,

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gently picked out of the tube with a small brush, placed in a Petri dish, and observed under a stereomicroscope. The different life stages (N1 to N5) were identified by observing morphological features (as in Vidano, 1964). Once determined, the nymphs were put back into the cages for them to complete their development, whereas the adults were permanently removed.

2.2. Statistical analyses and model building

The mean time of embryonic development, the mean embryonic developmental rate, the hatchability (% of eggs hatched on the total estimated with counts on the subsample), and the cumulative frequency of hatchings over time were calculated under each of the temperature regimes tested. Concerning post-embryonic development, for each of the temperatures and moults tested, frequency of moults over time, weighted mean and standard error, median, minimum, maximum, first and third quartile of the time of development (days), and survival rate were calculated. The data of insect mortality for each of the temperatures studied and moults tested were fitted to a 2nd order model ($y=ax^2 + bx + c$), to identify the optimal temperature for survival. This condition is satisfied when the 1st derivative of the model ($y'=2ax + b$) is equal to 0.

The developmental rate of *S. titanus* was modelled as a function of temperature. The experimental data were fitted to the non-linear equation of Brière et al. (1999):

$$RD\,(T) = n\,T\,(T - T_0)\,(T_L - T)^{1/m} \quad (1)$$

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where *RD* is the rate of development, expressed as the reciprocal of the number of days between phases, as a function of temperature *T*; T_L and T_0 are the upper (lethal) and lower cardinal temperatures, that is the temperature developmental thresholds ($T_0 \leq T \leq T_L$); and *n* and *m* are empirical constants.

As for T=15° C no data aside from those for eggs were obtained (see results), T_0 was estimated by regressing the reciprocal of developmental time in days (*y*) against temperature (*x*), and then solving the regression equation for *y*=0 (Rapagnani et al., 1988). In order to find the best fit, linear ($y = a\ x + b$), logarhitmic ($y = a\ ln\ x + b$), and inverse ($y = a/x + b$) regression equations were compared. The best fit was obtained with the inverse equation ($R^2 \geq 0.97$, $P < 0.05$), that was subsequently used for estimating T_0 for each of the moults. On the other hand, T_L was fixed at 40°C, after Rigamonti et al. (2011), as its experimental determination is problematic due to high mortality occurring at this temperature limit, resulting in selection of resistant (and thus not representative) individuals; moreover, the mortality at a certain constant temperature cannot exist if such a temperature is maintained for just a few hours.

The agreement between the observed and estimated values of the non-linear equation of Brière for each stage tested was based on the Mean Absolute Error (MAE), the Relative Root Mean Square Error (RRMSE), the Efficiency index (EF), and the Coefficient of Residual Mass (CRM) index (Loague and Green, 1991). The MAE measures how close predictions are to the observed data, and ranges from 0 (best) and +∞. The same range occurs in RRMSE. The EF index (that ranges from -∞ to 1) identifies inefficient models, as negative values indicate that the mean of all measures is a better predictor than the model used. The CRM indicates the tendency of the model to overestimate (if negative) or underestimate (if positive) the observed data. A perfect fit between observed and simulated data should result in MAE=0, RRMSE=0, EF=1, and CRM=0.

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The model was calibrated and validated with two independent field data sets, consisting in weekly visual inspections of the nymphal presence on grapevine leaves from the middle of May to the middle of July (details of the sampling method are given in Lessio and Alma, 2006) and monitoring the adults with yellow sticky traps from end June to end October. Samplings occurred in many vineyards throughout different vine growing areas of Piedmont (a total of 130, from 2006 to 2013), and were made partially by the authors, and partially by the Plant Protection Service of Piedmont for the application of pest management strategies. The mean hourly temperatures for each of the sampling points throughout different years were obtained via the meteorological station network of Piedmont (Regione Piemonte. Rete Agrometeorologica regionale e banca dati). For each year and meteorological station, the day of year (DOY) of the first appearance of each life stage of *S. titanus* was recorded. The data set was divided into a calibration and a validation subset, and thereafter the data of three meteorological stations were used for calibration, whereas 31 stations were used for validation. Two data sets of 18 and 69 observations were used for calibration and validation, respectively. For each life stage, at least three sampling units were used.

An appropriate script that calculates cumulative Brière's units (the dependent variable in eq. 1) for each of the stages of *S. titanus* as a function of mean hourly temperature was developed using the software R (http://www.r-project.org/). During calibration, the script was started from the date of the first observation of each of the life stages (obtained with field data). Concerning eggs, the calculation was started from January 1 (DOY=1), whereas the development of N1 and N4 was not subject to calibration due to insufficient data, and their appearance was forced based on single field-observations, on 5 May (DOY=124) for N1, and 19 June (DOY=169) for N4. Afterwards, calibration was made with an attempt-error procedure, adjusting the starting date to maximize the values of R^2 and to minimize RRMSE between the estimated and observed values of the appearance of each stage (given in DOY). This resulted in a different

value of Brière's units reached by each stage matching the start of the appearance of the next stage. The subsequent validation was made with the independent data set previously described. The error indices MAE, RRMSE, EF, CRM, and the correlation coefficient R^2 between the observed and predicted DOY values were calculated for both calibration and validation.

Finally, a linear regression was performed between the observed and predicted DOY values, without taking into account the different life stages, to estimate the overall model performances. As well, a homogeneity of regression test was made between the obtained regression line and the bisector ($y=x$), to determine if there were any under or overestimation of the observed data.

All statistical analyses were carried out with the Curve Expert Professional Software, version 1.5.0 (Hyams, 2012).

3. Results

3.1. Egg-hatching patterns

Eggs of *S. titanus* were detected in all the sub-samples of grapevine wood inspected. Out of 30 pieces of wood, 118 eggs were found (mean 3.90, standard deviation 2.07, minimum 2, maximum 9 eggs/piece). Therefore, the estimated number of eggs per Kg of wood was approximately 330, and this mean value was multiplied by the weight of the wood inside the rearing cage to estimate the initial number of eggs.

The post-dormancy (i.e. the time between the displacement of the wood in the climatic chambers and the start of hatching) varied along with temperature, as at T=24° it took 24 days, at T=20° and 22° about 42-47 days, respectively (Fig. 1). The cumulated frequency of hatched eggs reached 50% after 60, 75, and 50 days from the start (post-dormancy included) for T=20°, 22°,

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and 24°, respectively; whereas the hatchings stopped after 75 days with T=20° and 24°, and after 90 days with T=22° (Fig. 1). No hatching was observed for T=15° (data not shown) and 18°, whereas the maximum hatchability (64.58%) was recorded with T=22°. Hatchability was about 52% at T=20°, and 59% for T=24°; at 27° and 29°, only one and five eggs hatched, respectively. The mean developmental time (including post-dormancy) was negatively related to rising temperatures up to 24° (38 days), whereas it increased again at T=29°; however for such a temperature too few eggs hatched (Table 1).

3.2. Post-embryonic development

The time of development (T.D.) at the chosen temperatures was calculated for all the moults of *S. titanus*. The only exception was T=15°, under which too few nymphs survived (≈ 90% mortality, data not shown); besides, the grapevine plant itself started to shed the leaves and entered a dormancy phase. At T ≥ 18°, the developmental rate clearly increased along with temperature in all instars (Fig. 2); the overall post-embryonic developmental time ranged from 21 days at 29° to 52.8 days at 18° (Fig. 2).

The frequency distribution of moults occurring at different temperatures is shown in figure 3, whereas the descriptive statistics (mean, standard error, median, first and third quartile) of the duration of the post-embryonic development of *S. titanus* are listed in figure 4. As a result, within the same temperature the later instars took more time for development than earlier ones. For instance, at T=24° N1 to N2 took only five days, whereas N4 to N5 and N5 to adult took seven and eight days, respectively. Again, at the extreme T=29°, N1 to N2 took approximately three days, and N5 to adult more than five days. The highest mortalities were recorded at the limit temperatures of 18 and 29°, and were higher for early than for late instars; on the other hand, the observed mortality was generally minimal at 24°. N1 survived quite well between 22°

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and 27° (approx. 5-12% of mortality) whereas they were affected by lower (23-33%) and higher (29%) temperatures. N2 showed a similar pattern, although the survival between 24° and 27° was slightly lower, and the highest mortality (33%) was noted at the extreme temperature of 29°. N3 survived fairly well between 20° and 25°, whereas many of them (41-43%) died at the opposite extreme temperatures. N4 showed the highest absolute mortality of all instars (44.5%) at 18°, whereas they survived better than N3 at T≥24°C. Finally, N5 were similar to N4 in extreme temperature values, whereas they were less fit at 22-24° C (Table 2). The 2nd order polynomial model produced an excellent fit with experimental data, and the predicted mortality was minimal between 23° and 25°C (Table 3).

3.3. Model fitting

Brière's curves obtained for the different post-embryonic life stages of *S. titanus* showed different slopes (*1/m*), indicating different increases of developmental rates, and the highest slope was recorded for N2 (m=0.81), and the lowest for N4 (m=1.46). The minimum development temperature (T_0) ranged from 12.4° C (N4) to 14.5° (N2). Optimum temperatures, T_{opt} (which maximize the predicted developmental rate, that is, when the first derivative of the Brière's function is equal to zero) were 28.5-31.8° C (Table 4, Fig. 5). On the other hand, the inflection point of post-embryonic development (when the increase starts to be less than proportional) was always reached with T≈22° C (Fig. 5).

Concerning eggs, the minimum temperature T_0=18° C was obtained experimentally, whereas T_L was obtained via interpolation at T=30° C. The rate of development increased quite quickly up to 24°, and the maximum (T_{opt}) was at 25.7° C. The response at T=27° C, however, should be considered carefully because of the low number of eggs hatched (Table 4, Fig. 5).

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The agreement between observed and estimated values of the non-linear equation of Brière, for each stage tested, is shown in Table 5. The four common indices of prediction error in time series analysis confirmed the high reliability of the model used, with R^2>0.90 and P<0.01. The MAE was close to the optimal value of 0. The EF, ranging 0.87-0.96, indicates a good predictive power of the model compared to the sample mean. The values of analysis of the RRMSE were between 6.8 (for N2) and 12.3 (for N1), and indicate that the percentage error of the model is always less than 13%. Finally, the CRM was always positive but close to the optimum 0, indicating a good estimation of the model compared to the average value.

During the calibration phase, fairly good R^2 values were obtained for N2 and N3, but without significance. On the other hand, R^2 was significant for adults. The MAEs and RRMSEs were quite low for N2, N3 and N5, indicating an error of 4-6 days between the observed and predicted DOY; for the same stages, and for adults, the CRM values were close to the optimum zero but they were always positive, that indicates a slight tendency of the model to underestimation. EF values were negative, and ranged from -0.41 to -1.57. Concerning adults, higher MAE and RRMSE values were obtained (6.26 days and 10.33 %, respectively), whereas EF was -7.94 (Table 6).

Validation results showed significant R^2 values only for N3 and adults; RRMSE and MAE ranged 1.6-4.6 % and 2.0-4.9 days, respectively, with the exception of N1 and N5 that produced very high errors. The EF values were positive for N3, N4 and adults, and negative for the other stages. Again, CRM values were positive and indicated a slight underestimation, but they were close to the optimum zero (Table 6).

The linear regression between the overall observed and predicted DOY values was significant (R^2=0.67, P<0.001), indicating a good performance of the model proposed (Fig. 6). The homogeneity of regression test between the regression line and the bisector ($y=x$) was not

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significant (F=2.02, df=1, 135, P=0.16), indicating no under or overestimation of the observed data.

4. Discussion

4.1. Egg-hatching patterns

One of the novel contributions of this research was the calculation of the minimum (T_0=18°) and maximum (T_L=27°) cardinal temperatures for *S. titanus* eggs. These parameters, along with the thermal requirement for stopping dormancy stated by Chuche and Thiéry (2009), could be important in the future in order to determine the potential geographical distribution of this leafhopper. In fact, the thermal requirements of overwintering stages are often critical for geographical distribution. For instance, Gutierrez et al. (2012) simulated the distribution of the European grapevine moth, *Lobesia botrana* (Denis & Schiffermuller) in California as a function of temperature, based upon the thermal requirements of certain life stages such as overwintering pupae. *Scaphoideus titanus* overwinters in the egg stage, and the duration of dormancy depends on winter temperatures since hatching is delayed if eggs are exposed to mild rather than cold winters (Chuche and Thiéry, 2009). However, as our egg source came from the same lot of grapevine wood, the difference in hatching duration should depend just on the different temperature regimes (from 20° to 27°) the eggs were exposed to. The duration of the post-dormancy period was minimum at 24° (38 days), that is 235.6 degree-days if considering a $T_0 \approx$ 18°. Our data are in accordance with those reported by Rigamonti et al. (2011), who calculated it to be about 196 degree-days by collecting N1 with beating trays in the vineyards.

Under laboratory conditions at constant temperatures, hatching duration was in accordance with observations in the open field (Vidano, 1964; Rigamonti et al., 2011). Hatchability was

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minimum at high rather than low temperatures: this aspect is probably due to the fact that *S. titanus* originates from temperate and not tropical regions (Barnett, 1976), and overwinters in the egg-stage (Vidano, 1964), and may be therefore more affected by high than low extreme temperature regimes. However, these data should be considered carefully as the initial number of eggs was estimated and not observed. Hatching length is also influenced by winter temperatures, being shorter after cold winters and vice versa (Chuche and Thiéry, 2014). From a biological point of view, a different length of the hatching period may be considered an adaptation of the insect to the phenology of grapes, as nymphs need to find a suitable number of developed leaves in order to feed and find shelter. From a pest management point of view, a late hatching may result in nymphs that originate adults at the end of the season, with a further risk of phytoplasma transmission. As the insecticidal treatments to apply in the vineyards must take into account the residuals in grapes, the knowledge of egg hatching patterns is important to maximize pest management effects, for example targeting as many specimens of the same instar as possible with a single spraying event.

As for other leafhopper species, *Erythroneura ziczac* Walsh shows lower developmental times (=higher developmental rates) at the same temperatures tested (Olsen et al., 1998); *Cicadulina bipunctata* (Melichar) has a very shorter developmental period of eggs (Tokuda and Matsumura, 2005); Knight et al. (1991) tested the developmental rate of *Typhlocyba pomaria* McAtee eggs at T=12°, 16°, 20°, and 25°, and found a slight increase along with rising temperatures, but they claimed that the optimum egg-hatching temperature was probably above 25°; and finally, Tokuda and Matsumura (2005) found that the developmental time for eggs of *C. bipunctata* constantly decreases along with increasing temperatures from 16° to 34°, but with a significantly lower survivorship at 16°. In *S. titanus,* the cumulative frequency of hatchings reached 50% after 60, 75 and 50 days from the start of rearing at 20°, 22° and 24°, respectively. Knight et al. (1991) found that the simulated cumulative egg hatching of *T. pomaria* in the open field reached 100%

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in 30 days: the difference between this species and *S. titanus* may be due to the different size of eggs.

4.2. Post-embryonic development

The dynamics of age structures in *S. titanus* was clearly related to temperature, as within the same instar, the development was faster at increasing temperatures. As a result, the whole post-embryonic development of *S. titanus* should last about one month at a constant temperature of 24°. According to field data, the life cycle from N1 to adults lasts about 50 days (Vidano, 1964). The mean daily temperatures of May and June, in the main vine-growing areas of North-western Italy, are about 17-18°C and 20-21°C, respectively (Barni et al., 2012); in these conditions, the life cycle of *S. titanus* should match the laboratory data obtained during this research. However, the developmental rate may be different under constant or variable temperature conditions, due to rate summation effects (Worner, 1992; Liu et al., 1995). Another issue to be addressed is micro-climate, in the measure that nymphs stay on the lower leaf surface (Vidano, 1964), where temperature values are lower than those registered in a meteorological station. On the other hand, insects placed inside a rearing cage under constant temperature conditions cannot choose a cooler environment. This may explain the shorter life cycle of *S. titanus* obtained in the laboratory at constant temperatures, compared to what happens in the open field at similar mean temperatures when the different life stages appear. Finally, the duration of the whole life cycle of an insect is often different than the one calculated as a summation of the durations of different life stages, and many factors besides temperature (such as relative humidity, food sources, etc.) can increase and/or decrease the developmental time (Danks, 2000).

Concerning other leafhoppers, post-embryonic development is often strongly related to temperature, both if calculated under constant or variable (=field) conditions (Fielding et al.,

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1999). *C. bipunctata* nymphs took a shorter period of time for completing their development, about 22 days for T=22° and 15 days for T=25° (Tokuda and Matsumura, 2005), whereas the whole post-embryonic development of *S. titanus* took 26 and 22 days for the same temperatures, respectively. The same happened for *E. ziczac* (Olsen et al, 1998), which developed faster than *S. titanus*. On the other hand, *Graminella nigrifrons* (Forbes) displayed values comparable to those of *S. titanus* (Sedlacek et al., 1990). This could be due to the fact that *C. bipunctata* and *E. ziczac* are smaller than *S. titanus* and, therefore, need a shorter period of time for developing at the same temperatures, whereas *G. nigrifrons,* which has similar developmental rates, is about the same size. Besides the different developmental times, however, the response of *S. titanus* nymphs to temperature matches those of other leafhopper species. For instance, Sedlacek et al. (1990) found a linear developmental rate in *G. nigrifrons* between 18° and 30°. On the other hand, Knight et al. (1991) found a non-linear development function for nymphs of *T. pomaria* between 25° and 30°, whereas in a similar range of temperatures (24-29°) the response of *S. titanus* was almost linear.

At the same temperature, the development was faster for earlier rather than later instars. In many species of arthropods the temperature requirements for later instars are higher than for earlier ones (Danks, 2000), and body size has an effect on development time as well (Gillooly et al., 2002). As later instars of *S. titanus* appear when air temperatures are higher, however, the duration of the different instars may not differ substantially along the season. This may be the same reason for the shorter time of development displayed by some Typhlocybinae, at the same temperature (Olsen et al., 1998; Tokuda and Matsumura, 2005). Note that members of this family are quite small in size, whereas, for instance, *Graminella nigrifrons* (Forbes) (Deltocephalinae) displayed values comparable to those of *S. titanus* (Sedlacek et al., 1990).

The mortality of *S. titanus* nymphs was always minimum for T=24°C, and later instars (N3-N5) suffered from low rather than high extreme temperatures, probably because larger species (and

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therefore also larger instars of the same species) have higher thermal requirements and can better tolerate higher than lower temperatures (Danks, 2000; Gillooly et al., 2002). Our estimation of *S. titanus* survival via regression analysis at constant temperature regimes is in accordance with Régnière et al. (2012). This last aspect is in accordance with N3 appearing in the second half of June (Vidano, 1964), when in Northwestern Italy mean daily temperatures are about 24°C, and max. temperatures can reach 35°-37° C (Brunetti et al., 2005). Therefore, a decrease in temperature during the late instar period (that is, between the second half of June and the first half of July) may cause some mortality in *S. titanus*. However, other factors besides temperature must be taken into account in calculating the field mortality in *S. titanus* nymphal instars.

The survivorship of *S. titanus* nymphs was quite high if compared to those of *C. bipunctata*, reared at the same temperatures on rice seedlings (Tokuda and Matsumura, 2005). This discrepancy may be due to the different physiology of host plants, or to the rearing method: *C. bipunctata* was reared on rice seedlings inside small glass tubes, whereas the cages with a potted grapevine where we put *S. titanus* nymphs may be closer to natural conditions.

4.3. Model fitting and validation

During parametrization the Brière model provided a good fit of the experimental data, with high R^2 values and low errors. Although it was not possible to determine experimentally the extreme temperatures T_0 and T_L for post-embryonic development, these extreme values are not reached for more than 3-4 hours per day in north-western Italy (Brunetti et al., 2005). Thus the estimation of extreme values from the literature and *via* interpolation appears to be sound. According to Régnière et al. (2012), upper and lower critical temperatures are one of the main issues to be addressed when building a phenological model. Rigamonti et al. (2011) tested the equation of Brière for the whole nymphal developmental time, and found T_0=8.7°, whereas the present

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research suggest that this value is higher, between 12° C and 14°C, for late instars (N4-N5); again, the discrepancy may be due to the difference of modelling under constant or variable temperature conditions (Worner, 1992; Liu et al., 1995), or because the two models are obtained from different (laboratory, and field) data sources.

Although care must be taken when modelling at extreme values, outside the range of experimental data (Liu et al. 1995; Danks, 2000), the choice of Brière's function was successful, and confirms the suitability of this model to depict the influence of temperature on the developmental rate of insects (Shi and Ge, 2010). The validation of the model was satisfactory for N3 and adults, which are the two key life instars for targeting IPM. Low errors were obtained also for N2 and N4, whereas for N1 and N5 errors where higher. Concerning N1, the poor performance may be due to a delay in estimation of the appearance during field sampling, which is very time-consuming when *S. titanus* is present at very low densities (the sampling plan used can detect a density of 0.02 nymphs/5 leaves per plant after having counted up to 137 plants and found 2 nymphs, with a 25% error rate) (Lessio and Alma, 2006), so *S. titanus* may have been present during early samplings but not detected. On the other hand, the appearance of N5 may have been influenced by insecticidal sprays that targeted the previous instars, causing as well a delay in the estimation in the field. However, an overall good performance of the model applied to field data was confirmed by the significant fit between the predicted and observed DOY values. Finally, the homogeneity of regression test proved how the model does not under or overestimate significantly the observed field data.

4.4. Conclusions and perspectives

The embryonic and post-embryonic development of *S. titanus* is strongly related to temperature, and the trend obtained under laboratory conditions is sound with the occurrence of the different

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life stages of this leafhopper in the open field. The parametrization of Brière's equation through laboratory data and its subsequent validation permitted to model the whole life cycle of this insect vector in Northern Italy. The possible income of the model proposed is the application to IPM in order to identify the best moment (that is, the maximum seasonal frequency of moults from N2 to N3, and from N5 to adults) for insecticide sprays. For instance, the sprays against nymphs (N3-N4) may better focus on the peak of presence in the field (that is, when the cumulated frequency equal to 50%), in order to target as many of them as possible. The understanding of the dynamics of age structure is very important in organic farming, as pyrethrum, the main active ingredients. used in Italy, is effective only against nymphs and must be applied several times per year (Lessio et al., 2011; Žežlina et al., 2013). Concerning adults, that are highly mobile and capable of transmitting phytoplasmas agents of FD, it would be better to apply insecticides at their first occurrence; moreover, in some regions a further insecticidal application is made in the later part of the season (August-September) against adults entering the vineyards from uncultivated areas, and in this case, it is important to know the distribution of late egg hatching and nymphal development in order to spray.

Forecasting models play a key role in pest management, as they permit to enhance the efficacy and timing of insecticidal sprays depending on weather data, saving resources and reducing environmental impact. The prediction of *S. titanus* seasonal dynamics is crucial for controlling the spread of FD, and within this frame the proposed phenological model should become a useful tool to guide pest management strategies against this insect vector.

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Table 1. Data of embryonic development of *Scaphoideus titanus* under different constant temperature conditions [a].

	Temperature (° C)					
	18	20	22	24	27	29
T.D. (mean ± s.e.)	0.0 ± 0.0	60.0 ± 10.1	56.7 ± 12.5	38.4 ± 15.4	34.0 ± 17.0	96.8 ± 13.2
D.R.	N.D.	0.02	0.03	0.03	0.03	0.01
Total E	144	355	248	636	251	205
Total N1	0	184	160	376	1	5
Mortality (%)	100.00	48.16	35.42	40.89	99.60	97.56

[a] T.D.: time of development (days); D.R.: developmental rate (days $^{-1}$); N.D.: not determined; total eggs (E) were estimated by proportion after having counted the number of eggs on a sub-sample of 25-30 pieces of grapevine wood (see text for details). N1: 1st instar nymphs.

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Table 2. Data of *Scaphoideus titanus* mortality (range 0-1) during moults at different constant temperatures [a].

T (°C)	N1	N2	N3	N4	N5
18	0.33	0.28	0.41	0.45	0.41
20	0.23	0.23	0.25	0.31	0.31
22	0.12	0.14	0.22	0.2	0.29
24	0.06	0.13	0.16	0.08	0.20
27	0.07	0.11	0.34	0.26	0.24
29	0.29	0.33	0.43	0.35	0.37

[a] N1-N5: nymphs (=juveniles) from 1st to 5th instar.

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Table 3. Results of the 2nd order polynomial regression of *Scaphoideus titanus* mortality during moults as a function of temperature [a].

Life stage	*a*	*b*	*c*	R^2	$T_{opt.}$	*Min. mort.*
N1	0.01	-0.38	4.65	0.89	24.05	0.08
N2	0.01	-0.28	3.49	0.77	23.33	0.22
N3	0.01	-0.36	4.40	0.93	23.08	0.25
N4	0.01	-0.43	5.30	0.91	23.89	0.16
N5	0.01	-0.25	3.31	0.88	25.00	0.19

[a] T_{opt} : optimal temperature (° C) providing minimum mortality (Min. mort., range 0-1); N1-N5: nymphs (=juveniles) from 1st to 5th instar.

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Table 4. Estimation of the parameters *n* and *m* of Brière's equation representing the phenological development of *Scaphoideus titanus* [a].

Life stage	*n*	*m*	T_0	T_{opt}	T_L	R^2	*P*
E to N1	5.14×10^{-5}	1.44	17.8	25.7	30.0	0.93	0.00
N1 to N2	7.31×10^{-5}	1.14	13.2	30.3	40.0	0.99	0.00
N2 to N3	3.21×10^{-5}	0.81	14.5	28.5	40.0	0.99	0.00
N3 to N4	4.99×10^{-5}	1.04	13.5	29.8	40.0	0.99	0.01
N4 to N5	8.43×10^{-5}	1.46	12.4	31.8	40.0	0.98	0.01
N5 to A	3.65×10^{-5}	1.04	12.9	29.7	40.0	0.94	0.03

[a] T_0, T_{opt}, and T_L are lower, optimal and lethal temperature (° C) development thresholds, respectively. E: eggs; N1-N5: nymphs (=juveniles) from 1st to 5th instar; A: adults.

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Table 5. Estimation of the errors of Brière's equation fitted to experimental data of embryonic and post-embryonic development of *Scaphoideus titanus* [a].

Life stage	*MAE*	*RRMSE*	*EF*	*CRM*	R^2	*P*
E to N1	0.00	11.01	0.97	0.00	0.97	0.00
N1 to N2	0.02	12.33	0.83	0.02	0.86	0.01
N2 to N3	0.01	6.83	0.95	0.01	0.96	0.00
N3 to N4	0.01	7.90	0.93	0.01	0.95	0.00
N4 to N5	0.01	11.08	0.87	0.02	0.90	0.00
N5 to A	0.01	10.02	0.87	0.01	0.89	0.01

[a] MAE: mean absolute error; RRMSE: root mean square error; EF: efficiency index; CRM: coefficient of residual mass; E: eggs; N1-N5: nymphs (=juveniles) from 1st to 5th instar; A: adults

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Table 6. Results of calibration and validation of Brière's equation using experimental field data of appearance of the different life stages of *Scaphoideus titanus* [a].

Life stage	*N*	*BU*	DOY_{obs}	DOY_{pred}	R^2	*P*	*RRMSE*	*MAE*	*EF*	*CRM*
Calibration										
N2	6	44	148.5 ± 4.97	147.8 ± 11.32	0.64	0.06	4.90	5.67	-1.57	0.00
N3	6	41	165.7 ± 7.34	160.8 ± 10.53	0.57	0.08	4.81	6.17	-0.41	0.03
N5	3	33	177.7 ± 5.03	176.7 ± 9.50	0.33	0.61	3.63	5.67	-1.47	0.01
A	3	30	192.3 ± 4.93	190.0 ± 9.53	1.00	0.03	6.26	10.33	-7.94	0.01
Validation										
N1	7	2.3	151.29 ± 3.40	126.7 ± 6.87	0.35	0.16	16.59	24.57	-62.55	0.16
N2	16	44	161.81 ± 3.64	158.0 ± 4.43	0.17	0.11	3.62	4.94	-1.21	0.02
N3	28	41	166.32 ± 5.10	165.8 ± 5.34	0.39	0.00	2.71	3.89	0.19	0.00
N4	5	5	172.60 ± 9.53	169.8 ± 1.48	0.68	0.09	4.62	4.80	0.12	0.02
N5	3	35	186.00 ± 11.79	170.7 ± 10.26	0.25	0.67	11.75	15.33	-4.16	0.08
A	10	30	187.20 ± 3.68	186.8 ± 4.78	0.56	0.01	1.62	2.00	0.24	0.00

[a] N: number of observations used; BU: value of Brière's units that causes the start of calculation of the subsequent stage; DOY: day of year (mean ± standard deviation), observed in the field (obs) or predicted by the model (pred); RRMSE: relative root mean square error: MAE: mean absolute error; EF: efficiency index; CRM: coefficient of residual mass.

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Figure captions

Figure 1. Cumulative distribution frequency of egg hatching patterns of *S. titanus* as a function of time, under different constant temperature conditions.

Figure 2. Duration of development of the different life stages of *S. titanus* (mean ± standard error) under different constant temperature conditions. N1-N5: nymphs (=juveniles) from 1st to 5th instar.

Figure 3. Frequency distribution of moults of *S. titanus* as a function of time, under different constant temperature conditions. N1-N5: nymphs (=juveniles) from 1st to 5th instar.

Figure 4. Mean, standard error, median, first and third quartile of the duration of post-embryonic development for *S. titanus* under different constant temperature conditions. N1-N5: nymphs (=juveniles) from 1st to 5th instar.

Figure 5. Fitting of Brière's equation of the mean of the observed developmental rate (D.R., expressed as day^{-1}) for the different life stages of *S. titanus* as a function of temperature (line: predicted values; diamonds: observed values). E: eggs; N1-N5: nymphs (=juveniles) from 1st to 5th instar.

Figure 6. Linear regression between the observed and predicted days of year (DOY) of appearance of *S. titanus* life stages. N1-N5: nymphs (=juveniles) from 1st to 5th instar; A: adults.

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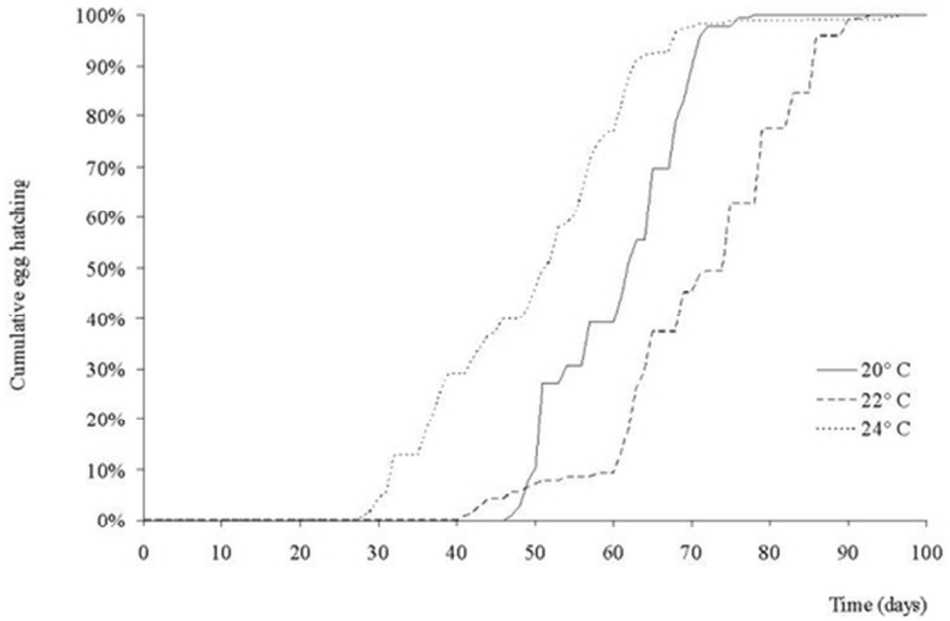

Cumulative distribution frequency of egg hatching patterns of S. titanus as a function of time, under different constant temperature conditions.
24x18mm (600 x 600 DPI)

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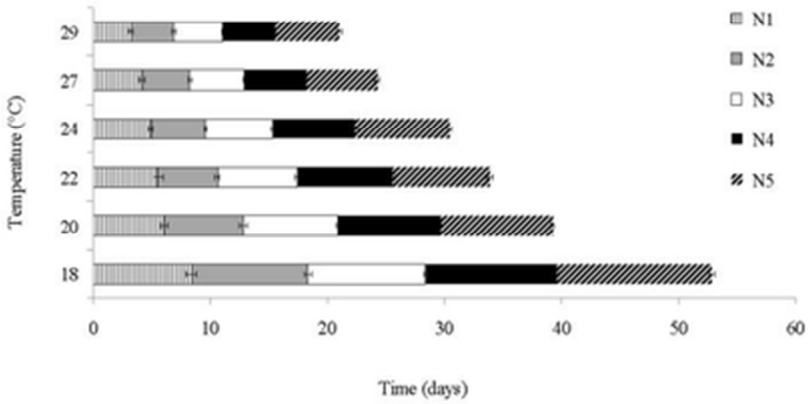

Duration of development of the different life stages of S. titanus (mean ± standard error) under different constant temperature conditions. N1-N5: nymphs (=juveniles) from 1st to 5th instar; A: adults.
19x11mm (600 x 600 DPI)

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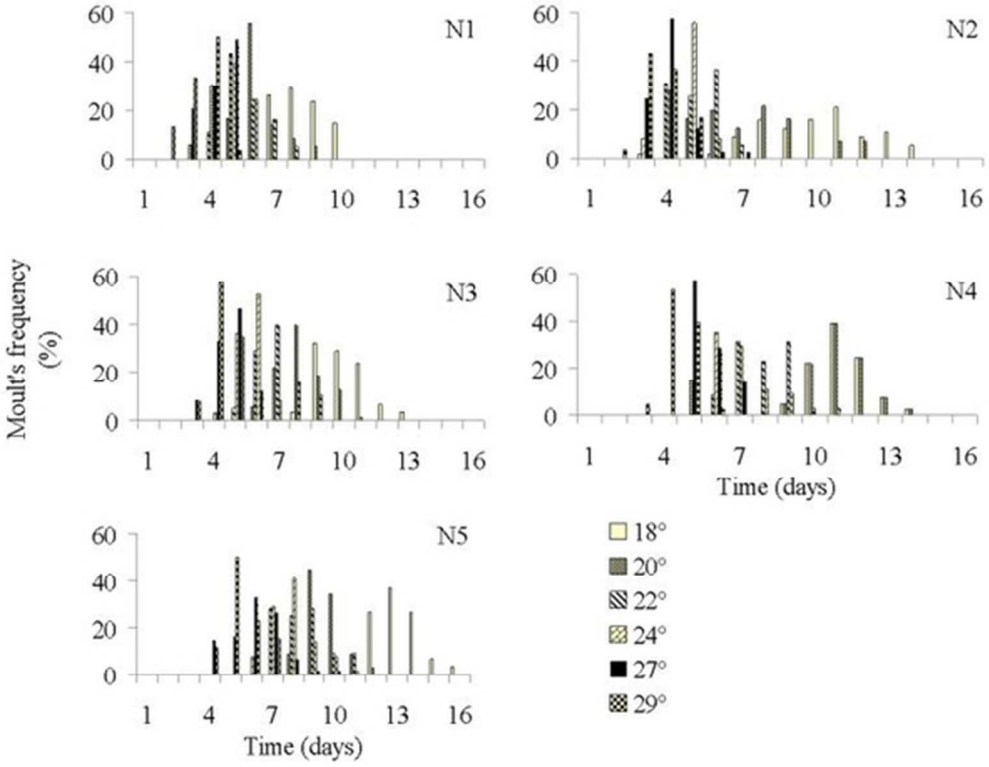

Frequency distribution of moults of S. titanus as a function of time, under different constant temperature conditions. N1-N5: nymphs (=juveniles) from 1st to 5th instar; A: adults.
25x19mm (600 x 600 DPI)

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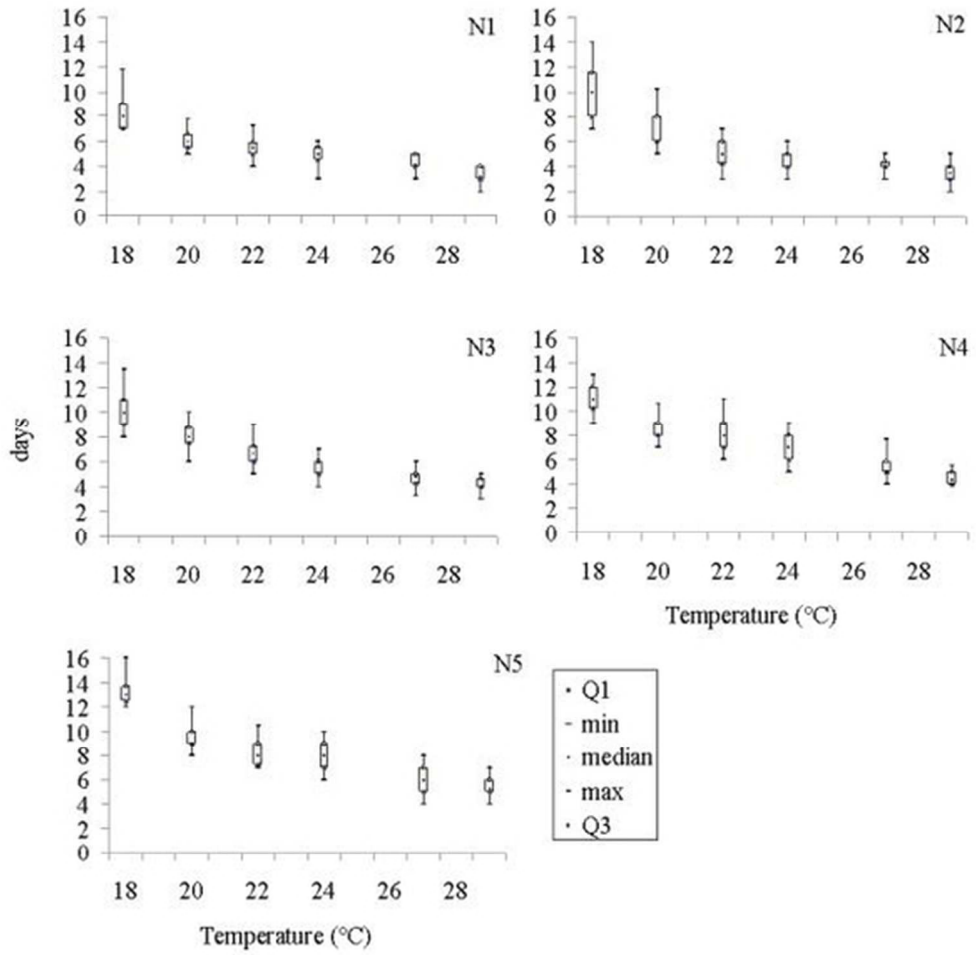
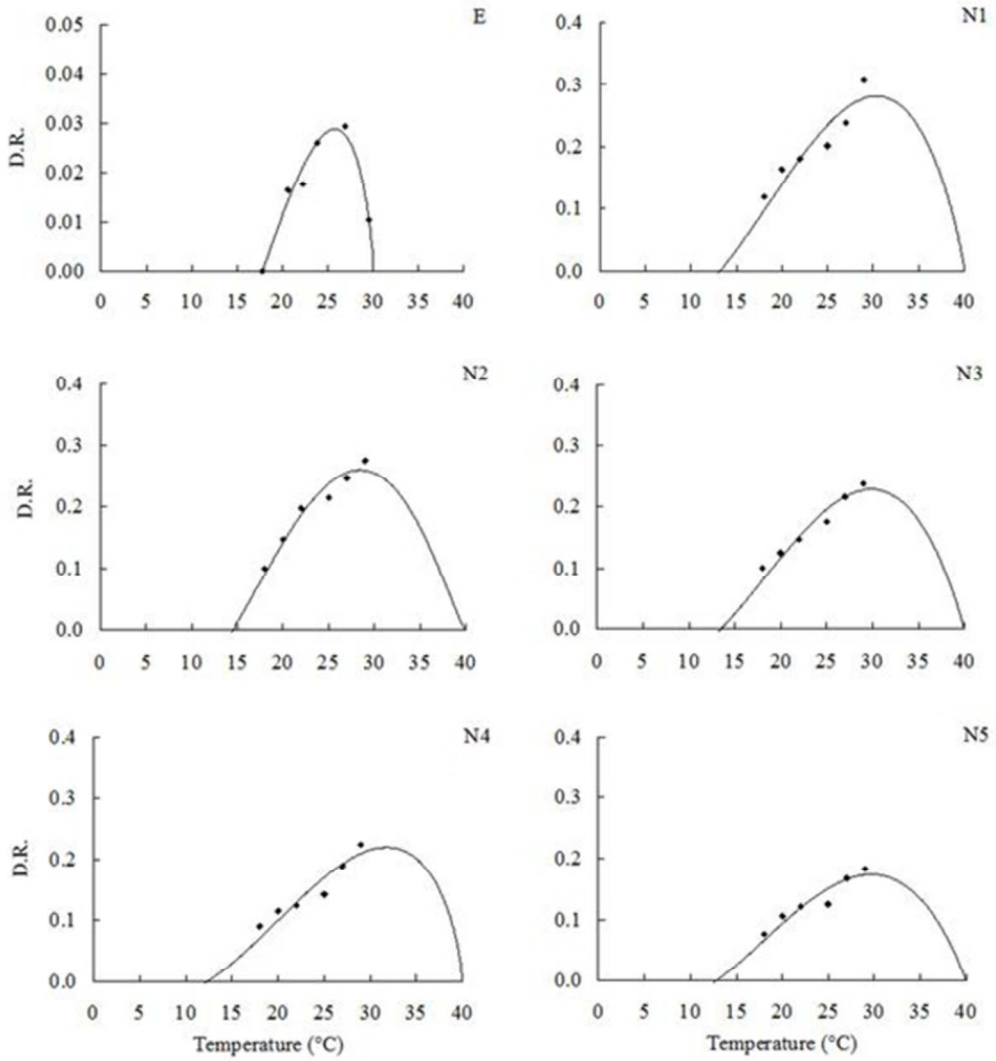
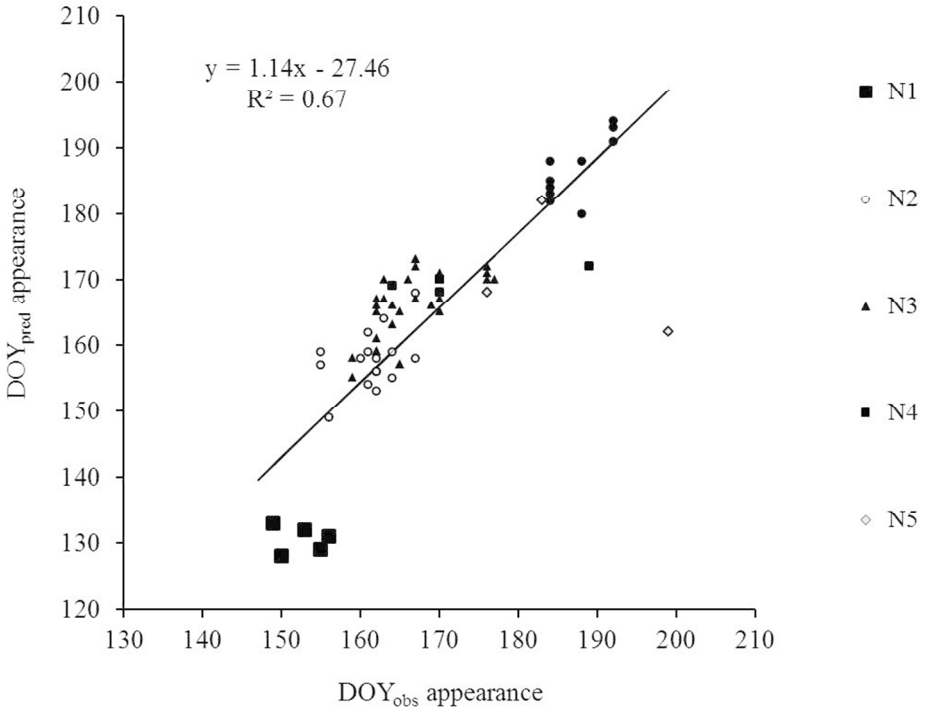


Mean, standard error, median, first and third quartile of the duration of post-embryonic development for S. titanus under different constant temperature conditions. N1-N5: nymphs (=juveniles) from 1st to 5th instar; A: adults.
25x24mm (600 x 600 DPI)



Fitting of Brière's equation of the mean of the observed developmental rate (D.R., expressed as day-1) for the different life stages of S. titanus as a function of temperature (line: predicted values; diamonds: observed values). E: eggs; N1-N5: nymphs (=juveniles) from 1st to 5th instar.
27x29mm (600 x 600 DPI)



Linear regression between the observed and predicted days of year (DOY) of appearance of S. titanus life stages. N1-N5: nymphs (=juveniles) from 1st to 5th instar; A: adults.
182x134mm (150 x 150 DPI)

For Review Only